

CLAIMS

We claim:

1. A method for identifying strains of microorganisms comprising:
a) providing
i) a cleavage means; and
ii) a nucleic acid substrate containing sequences derived from one or more microorganism;
b) treating said nucleic acid substrate under conditions such that said substrate forms one or more cleavage structures; and
c) reacting said cleavage means with said cleavage structures so that one or more cleavage products are produced.

2. The method of Claim 1, wherein said cleavage means is an enzyme.

3. The method of Claim 2, wherein said enzyme is a nuclease.

4. The method of Claim 3, wherein said nuclease is selected from the group consisting of Cleavase™ BN, *Thermus aquaticus* DNA polymerase, *Thermus thermophilus* DNA polymerase, *Escherichia coli* Exo III, and the *Saccharomyces cerevisiae* Rad1/Rad10 complex.

5. The method of Claim 1, wherein said nucleic acid substrate comprises a nucleotide analog.

6. The method of Claim 5, wherein said nucleotide analog is selected from the group consisting of 7-deaza-dATP, 7-deaza-dGTP and dUTP.

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7. The method of Claim 1, wherein said nucleic acid of step (a) is substantially single-stranded.

8. The method of Claim 1, wherein said nucleic acid is RNA.

9. The method of Claim 1, wherein said nucleic acid is DNA.

10. The method of Claim 1, wherein said nucleic acid of step (a) is double stranded.

11. The method of Claim 10, wherein said treating of step (b) comprises:

i) rendering ^{the} said double-stranded nucleic acid ^{substrate} substantially single-stranded; and

ii) exposing ^{the} said single-stranded nucleic acid to conditions such that said single-stranded nucleic acid has secondary structure.

12. The method of Claim 11, wherein ^{the} said double-stranded nucleic acid is rendered substantially single-stranded by increased temperature.

15. The method of Claim 1, further comprising the step of detecting said one or more cleavage products.

14. The method of Claim 1, wherein said microorganism ^{reference} ^{is a bacterium} comprises bacteria.

15. The method of Claim 14 wherein said bacteria are selected from the group comprising members of the genera *Campylobacter*, *Escherichia*, *Mycobacterium*, *Salmonella*, *Shigella* and *Staphylococcus*.

15. 16. The method of Claim 15 wherein said members of the genus *Mycobacterium* comprise strains of multi-drug resistant *Mycobacterium tuberculosis*.

17. 16. The method of Claim 1 wherein said ^{reference} microorganism ~~comprises virus~~ is a bacterium.

18. 16. The method of Claim 17 wherein said virus is selected from the group ¹⁶ comprising hepatitis C virus and simian immunodeficiency virus.

19. A method for detecting and identifying strains of microorganisms, comprising:
a) extracting nucleic acid from a sample suspected of containing one or more microorganisms; and
b) contacting said extracted nucleic acid with a cleavage means under conditions such that said extracted nucleic acid forms one or more secondary structures, and said cleavage means cleaves said secondary structures to produce one or more cleavage products.

15 18. 19. The method of Claim 19 further comprising the step of separating said cleavage products.

20 23. 18. The method of Claim 19, further comprising the step of detecting said cleavage products.

22. 24. 23. The method of Claim 23, further comprising comparing ^{the} detected cleavage products generated from cleavage of said extracted nucleic acid isolated from said sample with separated cleavage products generated by cleavage of nucleic acids derived from one or more reference microorganisms.

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25. The method of Claim ~~19~~ further comprising the step of isolating a polymorphic locus from said extracted nucleic acid after the extraction of step a), to generate a nucleic acid substrate wherein said substrate is contacted with the cleavage means of step b.

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26 24. The method of Claim ~~23~~ wherein ~~said~~ isolation of a polymorphic locus is accomplished by polymerase chain reaction amplification.

27 25. The method of Claim ~~24~~ wherein said polymerase chain reaction is conducted in the presence of a nucleotide analog.

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28 26. The method of Claim ~~25~~, wherein said nucleotide analog is selected from the group ^{consisting of} ~~comprising~~ 7-deaza-dATP, 7-deaza-dGTP and dUTP.

29 27. The method of Claim ~~24~~ wherein said polymerase chain reaction amplification employs oligonucleotide primers matching or complementary to consensus gene sequences ~~derived~~ from said polymorphic locus.

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30 28. The method of Claim ~~25~~ wherein said polymorphic locus comprises a ribosomal RNA gene.

31 29. The method of Claim ~~28~~, wherein said ribosomal RNA gene is a 16S ribosomal RNA gene.

30. The method of Claim 19, wherein ~~said~~ cleavage means is an enzyme.

31. The method of Claim ~~30~~, wherein ~~said~~ enzyme is a nuclease.

32. The method of Claim 31, wherein said nuclease is selected from the group consisting of Cleavase™ BN, *Thermus aquaticus* DNA polymerase, *Thermus thermophilus* DNA polymerase, *Escherichia coli* Exo III, and the *Saccharomyces cerevisiae* Rad1/Rad10 complex.

²⁰ ¹⁸ 53. The method of Claim ¹⁹, wherein said ^{extracted} nucleic acid of step (a) is substantially single-stranded.

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34. The method of Claim 19, wherein said nucleic acid is RNA.

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35. The method of Claim 19, wherein said nucleic acid is DNA.

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36. The method of Claim 19, wherein said nucleic acid of step (a) is double
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10 stranded.

~~35~~ ³⁴ The method of Claim 36, wherein said treating of step (b) comprises:

i) rendering said double-stranded nucleic acid substantially single-stranded; and

ii) exposing ^{the} said single-stranded nucleic acid to conditions such that ^{the} said single-stranded nucleic acid has secondary structure.

R The method of Claim 37, wherein said double-stranded nucleic acid is rendered substantially single-stranded by increased temperature.

37. The method of Claim 18 wherein said microorganism ^{the} comprises
bacterium bacteria.

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40. The method of Claim ~~39~~ wherein said bacteria are selected from the group comprising members of the genera *Campylobacter*, *Escherichia*, *Mycobacterium*, *Salmonella*, *Shigella* and *Staphylococcus*.

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41. The method of Claim ~~40~~ wherein said members of the genus *Mycobacterium* comprise strains of multi-drug resistant *Mycobacterium tuberculosis*.

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42. The method of Claim ~~19~~ wherein ~~said~~ ^{*the*} microorganism ^{*comprises*} _{*is a*} virus.

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43. The method of Claim ~~42~~ wherein ~~said~~ ^{*the*} virus is selected from the group ^{*consisting of*} comprising hepatitis C virus and simian immunodeficiency virus.

- 10 44. A method for treating nucleic acid comprising an oligonucleotide containing microbial gene sequences, comprising:
- a) providing
 - i) a cleavage means in a solution containing manganese; and
 - ii) nucleic acid substrate containing microbial gene sequences;
 - b) treating said nucleic acid substrate with increased temperature such that said substrate is substantially single-stranded;
 - c) reducing said temperature under conditions such that said single-stranded substrate forms one or more cleavage structures;
 - d) reacting said cleavage means with said cleavage structures so that one or more cleavage products are produced; and
 - e) detecting said one or more cleavage products.